

Patent claims

1. An isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence which codes for the mikE17 gene, chosen from the group consisting of

5 a) polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,

10 b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70% to the amino acid sequence of SEQ ID No. 2,

15 c) polynucleotide which is complementary to the polynucleotides of a) or b), and

20 d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b) or c) ,
the polypeptide preferably having the activity of the transcription regulator MikE17.

2. A polynucleotide as claimed in claim 1, wherein the polynucleotide is a preferably recombinant DNA which is capable of replication in coryneform bacteria.

3. A polynucleotide as claimed in claim 1, wherein the polynucleotide is an RNA.

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4. A polynucleotide as claimed in claim 2, comprising the nucleic acid sequence as shown in SEQ ID No. 1.

5. A DNA as claimed in claim 2 which is capable of replication, comprising

30 (i) the nucleotide sequence shown in SEQ ID No. 1, or

5 (ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or

5 (iii) at least one sequence which hybridizes with the sequence complementary to sequence (i) or (ii), and optionally

5 (iv) sense mutations of neutral function in (i).

10 6. A DNA as claimed in claim 5 which is capable of replication,

10 wherein

10 the hybridization is carried out under a stringency corresponding to at most 2x SSC.

15 7. A polynucleotide sequence as claimed in claim 1, which codes for a polypeptide which comprises the amino acid sequences shown in SEQ ID No. 2.

15 8. A coryneform bacterium in which the mikE17 gene is attenuated, in particular eliminated.

15 9. The vector pCR2.1mikE17int,

20 9.1 the restriction map of which is reproduced in figure 1 and which

20 9.2 is deposited in the E.coli strain Top10/pCR2.1mikE17int under no. 14143 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ = German Collection of Microorganisms and Cell Cultures, Braunschweig) in accordance with the Budapest Treaty.

25 10. A process for the fermentative preparation of L-amino acids, in particular L-lysine, which comprises

30 carrying out the following steps:

10 a) fermentation of the coryneform bacteria which produce the desired L-amino acid and in which at least the mikE17 gene or nucleotide sequences which code for it are attenuated, in particular eliminated,

5 b) concentration of the L-amino acid in the medium or in the cells of the bacteria, and

c) isolation of the L-amino acid, the biomass and/or constituents of the fermentation broth optionally remaining in their entire amount or in portions in

10 the product obtained in this way.

11. A process as claimed in claim 10, wherein

bacteria in which further genes of the biosynthesis pathway of the desired L-amino acid are additionally enhanced are employed.

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12. A process as claimed in claim 10, wherein

bacteria in which the metabolic pathways which reduce the formation of the desired L-amino acid are at least partly eliminated are employed.

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13. A process as claimed in claim 10, wherein

the expression of the polynucleotide(s) which code(s) for the mikE17 gene is attenuated, in particular eliminated.

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14. A process as claimed in claim 10, wherein

the regulatory properties of the polypeptide (enzyme protein) for which the polynucleotide mikE17 codes are reduced.

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15. A process as claimed in claim 10, wherein

for the preparation of L-amino acids, coryneform microorganisms in which at the same time one or more of the genes chosen from the group consisting of

- 15.1 the dapA gene which codes for dihydridipicolinate synthase,
- 15.2 the gap gene which codes for glyceraldehyde 3-phosphate dehydrogenase,
- 15.3 the tpi gene which codes for triose phosphate isomerase,
- 15.4 the pgk gene which codes for 3-phosphoglycerate kinase,
- 15.5 the zwf gene which codes for glucose 6-phosphate dehydrogenase,
- 15.6 the pyc gene which codes for pyruvate carboxylase,
- 15.7 the mqo gene which codes for malate-quinone oxidoreductase,
- 15.8 the lysC gene which codes for a feed-back resistant aspartate kinase,
- 15.9 the lysE gene which codes for lysine export,
- 15.10 the hom gene which codes for homoserine dehydrogenase
- 15.11 the ilvA gene which codes for threonine dehydratase or the ilvA(Fbr) allele which codes for a feed back resistant threonine dehydratase,
- 15.12 the ilvBN gene which codes for acetohydroxy-acid synthase,

15.13 the *ilvD* gene which codes for dihydroxy-acid dehydratase,

15.14 the *zwa1* gene which codes for the *Zwa1* protein is or are enhanced or over-expressed are fermented.

5 16. A process as claimed in claim 10,
wherein

for the preparation of L-amino acids, coryneform microorganisms in which at the same time one or more of the genes chosen from the group consisting of

10 16.1 the *pck* gene which codes for phosphoenol pyruvate carboxykinase,

16.2 the *pgi* gene which codes for glucose 6-phosphate isomerase,

16.3 the *poxB* gene which codes for pyruvate oxidase

15 16.4 the *zwa2* gene which codes for the *Zwa2* protein is or are attenuated, in particular eliminated, are fermented.

17. A coryneform bacterium which contains a vector which carries parts of the polynucleotide as claimed in
20 claim 1, but at least 15 successive nucleotides of the sequence claimed.

18. A process as claimed in one or more of the preceding claims,

wherein

25 microorganisms of the species *Corynebacterium glutamicum* are employed.

19. A process for discovering RNA, cDNA and DNA in order to isolate nucleic acids, or polynucleotides or genes which code for the transcription regulator *MikE17* or

have a high similarity with the sequence of the mikE17
gene,
which comprises
employing the polynucleotide comprising the
5 polynucleotide sequences as claimed in claims 1, 2, 3
or 4 as hybridization probes.

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